Approach to a Problem of Bioremediation Oil-polluted Raised Bogs in the Western Siberia (Russia)

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Abstract

This paper has described cleaning of an impassable raised bog polluted with oil in the Northern part of the Western Siberia (Russia) with the oil-oxidizing bacterial preparation Rhoder and has shown that obtained results lead us to the development of a new bioremediation technology for such bogs with the use of aerobic-anaerobic microbial processes there. Huge raised oligotrofic bogs of the Western Siberia hold very important ecological function, supporting a biodiversity of all inhabitants of such bogs and having influence on a climate and quality of surface water. Application of traditional remediation technologies there often causes irreparable damage to such bogs, furthermore these technologies (milling, introduction a lot of lime and fertilizers) are impossible and technically and economically unprofitable. In 2011, bioaugmentation technology with the use of the Rhoder was applied in the Western Siberia for the restoration of an impassable bog accidentally polluted with crude oil because of corrosion of pipe line. Oil was partially gathered by a pump for silts and then the Rhoder was applied three times without milling and with the application of fertilizers and lime only. As a result, the level of oil pollution in the soil was decreased by 32%-98% depending on initial concentration of HC and a depth of oil penetration into the moss. It was observed that indigenous anaerobic microorganisms took part in decontamination of oil pollution in the impassable bog. The obtained results have served as incentive to development of a new bioremediation technology with the application of electron acceptors for intensification of oil degradation in the depth of the polluted layer of moss.

Keywords

Bog, Moss, Oil, Augmentation, Microorganisms, Preparation Rhoder, Oil Degradation

Introduction

The main oil production areas in Russia are situated in the Westtern Siberia, and in the same places where there are huge bogs polluted with oil. Application of remediation technologies, developed in Russia, on impassable bogs polluted with oil is almost impossible technically and economically unfavorable. Besides a severe climate with cold and long winters and short cool summers, it is caused also by the absence of any road in tundra and forest-tundra as well as by emergency oil spills on raised bogs impassable for special machinery devices. A depth of oil penetration on bogs doesn't exceed 0.2-0.5 m (Kurchenko 1999) and is often propped up with water or permafrost. The oil pollution can extend on width and the irreparable damage will be caused to the Nature of this Region. Processes of self-restoration of such bogs can be prolonged for several hundred years. Therefore, huge raised oligotrofic bogs in the Western Siberia own very important ecological function, supporting a biodiversity of all inhabitants of such bogs and having influence on a climate and quality of surface water. So an elimination of such oil spills and their consequences on the bogs is a very actual and difficult problem.

In the Western Siberia in-situ bioremediation technology for the bogs polluted with oil is preferred because the excavation of a top layer of the moss polluted by crude oil from huge areas of impassable bogs is technically difficult and economically inefficient. Besides, the sheet of water with thickness about 3-10 m spreads under the moss layer on the most part of impassable bogs. With the best case, such bogs have once been milled at the very beginning of summer, until the permafrost layer was completely thawed. At once a large amount of fertilizers, lime, seeds of oats and any oil-oxidizing preparation is brought into the moss (Murygina, Arinbasarov, Kalyuzhnyi 1999). At worst, for example, behind the Polar Circle of the Western Siberia, the polluted bogs are left without any treatment. At the same time, the application of in-situ remediation technologies on raised bogs seldom happen successfully. Therefore,

the aim of this investigation is representation results of the bioremediation bog polluted with oil by using the Rhoder and also the reasons which propel us to be engaged in development of a new bioremediation technology which would be careful and solicitous one for raised bogs polluted with oil.

Material and Methods

The Oil-Oxidizing Preparation Rhoder

The Rhoder consists of two bacterial strains belonging to the genus Rhodococcus, (R. ruber Ac-1513 D and R. erythropolis Ac-1514 D), isolated from soils polluted with crude oil. The strains are non-pathogenic and non-mutagenic to humans, animals, plants and bacteria. The Rhoder is approved for wide use in the Nature and it has been successfully used for bioremediation of oil refinery sludge, soils, wetlands and water surfaces polluted with oil (Murygina, Korotaeva, Stolyarova, Peterson, Arinbasarov 1996; Murygina, Arinbasarov, Kalyuzhnyi 2000; Murygina, Markarova, Kalyuzhnyi 2005; Gally, Murygina, Kalyuzhnyi 2005; Ouyang, Yong-Yong, Liu, Murygina, Kalyuzhnyi, Zeng-De 2005; Ouyang, Liu, Yong-Yong, Murygina, Kalyuzhnyi, Zeng-De 2006; De-Qing, Jian, Zhao-Long, Jian, Tian-Li, Murygina, Kalyuzhnyi 2007) and the Rhoder is used in these described field-scale test.

The Western Siberia, Muravlenko Town

In 2011, an impassable bog located near the Muravlenko town with a size about 0.8 hectares (Figure 1) polluted with spring accidental oil spill because of corrosion of pipe line and halved by high knolls was offered for the bioremediation with using the Rhoder only Typical marsh plants (moss, cloudberry, wild rosemary) existed on the knolls, which were practically not affected by the oil spill. Large spots of the oil were situated on swampy impassable depressions. Vegetation (moss, sedge) on these depressions perished almost completely. A layer of the oil with a thickness about 1 cm and more was presented on the water surfaces on these depressions. While sampling, we discovered that an penetration of the oil into the moss was modified from 25 to 40 cm. The oil contamination of the bog was unequal. The bog had a slight bias towards a sand bank which had been made to prevent spreading of the oil pollution and, in fact, turned into the road. Two previously digging pits to collect oil with the pump were presented on the bog. However, oil was gathered poorly, and these pits still had much of oil. The thickness of the oil on the water surfaces of the pits were more than 1 cm as measured. The oil at an air temperature below 10^{o}C became viscous on the surface of the water of the depressions and pits.

The oil from the surface of the polluted bog was not collected additionally. The soil was not mixed by a disk harrow or other devices. An attempt to perform the bioaugmentation with the Rhoder was undertaken without additional gathering of the oil and without application of milling because of technical complication of doing classical ex-situ remediation on the impassable bog polluted with oil. It was needed to minimize expenses on the bioremediation.

The weather during the bioremediation of this bog was not favorable, the air temperature did not exceed 10 - 14°C, and it was raining from time to time.

Bioremediation

The oil polluted bog was treated three times with intervals for 3 weeks by the working solution of the Rhoder with the MPN of hydrocarbon oxidizing cells of $1.0*10^{8}$ per 1 ml by the sprinkling from the fire-engine vehicle, previously washed with water. The Rhoder was used in total quantity of 120 kg as a liquid concentrate with HCO bacteria cells of $1.0*10^{11}$ cells/mL.

Sampling for Analysis

Moss samples were collected before and after the finishing of the bioremediation from 12 points of the bog contaminated with oil from the depths of 0-10cm and 10-25cm, and 25-40cm (by using GPS) for microbiological, chemical and agrochemical analysis, respectively, each sample was with the weight of about 150 g. Then a complex of chemical, agrochemical and microbiological analysis during bioremediation was carried out and a well-known gravimetric, GC, HPLC and colorimetric methods were employed.

Chemical and Agrochemical Analyses.

Each sample of moss, polluted with oil, from field-tests (with concentration of HC till 600 g/kg) was dried at 75^{o}C and extracted on a Sockslet device (with a ball refrigerator, a flask and an extractor of volume 100 ml and 30 ml, accordingly by Info Symas.ru) with boiling $CHCl_3$, and gravimetrically determined. Then each dry material extracted by chloroform was fractioned on a mini-column with silica gel (Diapak-C). Hydrocarbons (HC) in oil were analyzed by the gas chromatograph (GC) and HPLC. GC model was the KristalLuks 4000м (by company Metakhrom) with the NetChrom V2.1

program, with the column OV-101 length of 50 m and internal diameter of 0.22 mm, with thickness of the phase of 0.50 microns and FID detector, at the temperature of the detector 300°C and the evaporator temperature of 280°C. The gradient was from 80°C to 270°C and the velocity of raising temperature was 12° C per minute. Mixture of Undecane, Dodecane, Tetradecane, Hexadecane and Squalane were used as external standards in concentrations of 5 μg/μL for each substance (Drugov, Zenkevich, Rodin 2005).

HPLC analyses were carried out on Knauer HPLC with the ultra-violet detector, on the reversed-phase column of Diasfer 110-C18 for HPLC, and length of a column was 250 mm, diameter-4 mm, grain-5 microns. Samples for analyses on HPLC were prepared after drying of hexane fractions and following extraction with 1 mL of acetonitrile during 20 min under shaking and then analyzed. Phenantrene, Pyrene and Benzo(e)pyrene were used as external standards in concentrations of 10 μg/μL for each substance in acetonitrile (Alekseenok, Gerasimova, Mikhailik 2009).

Several samples (8 samples) of moss (No. 1, 2, 3, 12, 18, 22, 24, 26) from the bog in the Western Siberia, Muravlenko town, were excessively polluted with crude oil. The oil from these samples at first was extracted by chloroform (150 mL) in chemical flasks (each flask with a capacity of 400 mL) which were shaken for 30 minutes at room temperature. Received solutions of the oil were transferred to the other flasks through waterless sodium sulfate (Na_2SO_4) to remove remains of water. The chloroform was evaporated at 75°C. Each sample of the moss excessively polluted with oil was then extracted three times as described above. The chloroform extracts in flasks were heated at 105°C till a constant weight. Samples of the moss after oil extraction were dried at 75°C, weighed and the total oil was calculated per 1 kg of a dry moss. Chemical analyses of HC in all other samples and the dried samples of the moss after previous extraction by chloroform at room temperature were carried out by a gravimetric method with the use of the Sockslet device and column chromatography with Silica gel (Drugov, Zenkevich, Rodin 2005).

pH of each sample, humidity and the general total content of the available nitrogen and phosphorus were determined with colorimetric methods (Mineev Ed. 2001).

Microbiological Analyses

MPN of microorganisms was determined in samples of the moss by using tenfold dilutions and cultivations in Petri dishes on meat-peptone agar and selective agar nutrients for identification of ammonifying microorganisms, actinomicetes, pseudomonas, oligotrophic bacteria and micromycetes. MPN of anaerobic microorganisms (first of all SRB) in samples of the moss was determined on the liquid Postgate's medium (Netrusov Ed 2005).

Determination of MPN of oil-oxidizing microorganisms in samples of moss was made by means of the modified liquid Raymond's media with oil as a sole carbon source (g/l): Na_2CO_3 - 0.1; $CaCl_2$*6 H_2O - 0.01; $MnSO_4$*7 H_2O - 0.02; $FeSO_4$ - 0.01; Na_2HPO_4*12H_2O -4.0; KH_2PO_4 - 1.0; $MgSO_4$*7 H_2O - 0.2; NH_4Cl - 2.0; NaCl - 5.0; pH = 7.0 (Nazina, Rozanova, Belyayev, Ivanov 1988).

Results and Discussion

Bioremediation of the Impassible Bog in the Western Siberia

The allocated object was very strongly polluted with oil (FIGURE 1), and it was difficult to expect a big success in such situation. Nevertheless, it was made a decision to test an oil-oxidizing ability of the Rhoder in such extreme conditions with using in-situ bioaugmentation technology. On the other hand, it was necessary to be convinced that bioaugmentation with the Rhoder can initiate self-restoration process though it may be not as effective as the ex-situ technology.

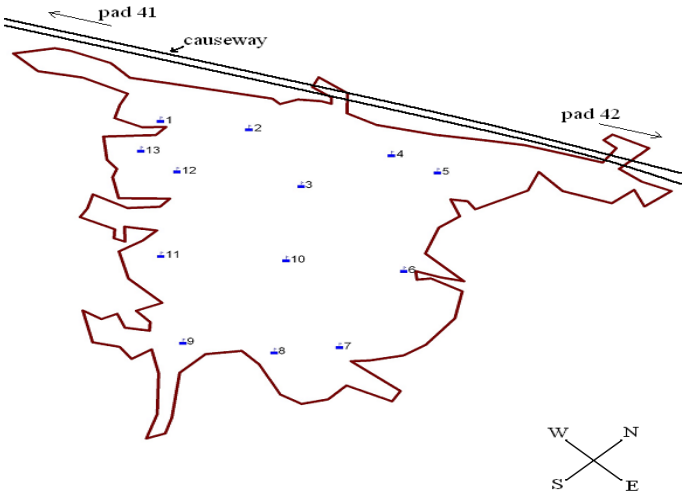

FIG 1. THE IMPASSABLE BOG POLLUTED WITH SPRING ACCIDENTAL OIL SPILL, THE WESTREN SIBERIA, MURAVLENKO, 2011

Microbiological Monitoring

Preliminary microbiological analysis of soil samples

showed (TABLE 1) that a lot of microorganisms were presented in layers of 0-10 cm. In these upper layers of the moss, the MPN of heterotrophic bacteria (HT) varied from $1.1*10^7$ to $6.1*10^8$ CFU/g of the moss. In these points, the level of the oil contamination varied from 60.3 g/kg DM to 903.6 g/kg DM. The MPN of HCO bacteria varied from $1.2*10^6$ cells/g to $1.1*10^8$cells/g of these mosses. In samples with a very high oil pollution the MPN of HCO cells was only $1.0*10^3$cells/g of the moss. In other samples taken from different depths of the bog, the MPN of HT and HCO microorganisms was lower (TABLE 1).

TABLE 1. MICROBIOLOGICAL AND AGROCHEMICAL CHARACTERISTICS OF MOSS SAMPLES BEFORE THE AUGMENTATION WITH THE RHODER

No	Depth of sampling	pH	HT CFU/g of moss	HCO, cells g of moss	$N\text{-}NH_4^+$ mg/kg of moss	PO_4^{3-} mg/kg of moss
1	(0-10)	-	-	-	-	-
	(10-25)					
2	(0-10)	5.2	$2.8*10^7$	$3.6*10^4$	5.08	-
	(10-25)	4.9	$2.5*10^7$	$4.3*10^4$	2.99	33.11
3	(0-10)	4.9	$6.1*10^8$	$8.1*10^7$	11.58	31.80
	(10-25)	5.0	$3.8*10^8$	$6.0*10^4$	9.18	22.28
4	(0-10)	5.4	$2.8*10^8$	$1.1*10^8$	17.40	33.53
	(0-25)	5.1	$6.1*10^7$	$3.8*10^7$	7.56	-
5	(0-10)	5.2	$1.1*10^7$	$4.9*10^7$	21.02	20.81
	(10-25)	5.0	$5.1*10^7$	$8.0*10^5$	15.91	-
	(25-40)	4.9	$1.8*10^7$	$7.9*10^5$	9.67	-
6	(0-10)	-	-	-	-	-
	(10-25)	5.	2.510^6	$6.0*10^4$	6.2	-
7	(0-10)	5.3	$1.9*10^7$	$8.4*10^4$	10.72	16.64
	(10-25)	4.9	$2.6*10^7$	$5.0*10^4$	16.01	-
8	(0-10)	4.9	$7.6*10^7$	$8.0*10^4$	11.14	19.10
	(10-25)	5.0	$6.4*10^6$	$7.7*10^3$	7.07	-
9	(0-10)	5.0	$1.1*10^8$	$7.1*10^3$	4.83	-
	(10-25)	4.9	$8.9*10^5$	$8.1*10^5$	7.56	-
10	(0-10)	5.1	$7.3*10^7$	$1.0*10^4$	6.35	-
	(10-25)	5.2	$2.8*10^6$	$1.0*10^7$	6.54	-
11	(0-10)	-	-	-	-	-
	(10-25)	5.0	$5.1*10^6$	$7.7*10^5$	3.30	-
12	(0-15)	-	-	-	-	-
	(15-30)	4.9	$5.9*10^7$	$1.2*10^6$	18.51*	-
13	(0-10)	-	-	-	-	-
	(10-25)	5.0	$6.1*10^7$	$9.6*10^4$	8.35	-

Note: - not detected because samples were unable to determine due to their high oil content or an insufficient amount of it

After three times introduction of the Rhoder, the total number of HT microorganisms as a whole didn't decrease and even increased in some samples by 1 order. The MPN of HCO bacteria increased by about 2 orders and more in the majority of the samples (TABLE 2). The negative influence of the oil-oxidizing preparation Rhoder on indigenous microorganisms wasn't observed.

TABLE 2. MICROBIOLOGICAL AND AGROCHEMICAL CHARACTERISTICS OF MOSS SAMPLES SELECTED AFTER AUGMENTATION WITH THE RHODER

No	Depth of sampling	pH	MPN of HT CFU/g of moss	MPN of HCO cells/g of moss	$N\text{-}NH_4^+$ mg/kg of moss	PO_4^{3-} mg/kg of moss
2	(0-10)	5.2	$2.8*10^7$	$4.4*10^6$	172.5	5.17
	(10-25)	6.3	$2.5*10^7$	$4.5*10^6$	99.6	8.84
3	(0-10)	6.4	$2.2*10^7$	$4.3*10^6$	284.9	9.98
	(10-25)	6.3	$3.8*10^8$	$5.1*10^6$	66.0	9.46
4	(0-10)	5.7	$1.7*10^7$	$5.9*10^7$	275.8	12.46
	(0-25)	5.8	$7.1*10^6$	$8.9*10^6$	163.3	2.60
5	(0-10)	-	-	-	-	-
	(10-25)	6.0	$2.4*10^6$	$3.7*10^6$	88.8	3.55
7	(0-10)	-	-	-	-	-
	(10-25)	6.0	$3.2*10^7$	$1.1*10^6$	139.9	3.58
8	(0-10)	6.3	$2.4*10^7$	$6.6*10^6$	240.7	2.15
	(10-25)	4.9	$1.8*10^7$	$1.0*10^6$	71.0	1.83

Note: - not analyzed

HC Degradation

Some samples of the moss selected for the preliminary examination of the bog were visually represented by oil slightly contaminated with moss. Several samples were examined relatively non-polluted, while the others were moderately polluted. 27 samples were selected from the different depths of the bog and analyzed before the bioremediation of this bog with the Rhoder.

On the right side of the bog in some places, the preliminary concentration of the crude oil in the moss layers of 0-10c m was from 35.13 to 14.35 kg/kg DM and residual concentration of HC in the same samples after extraction of the crude oil at the room temperature was from 290.6 to 66.9 g/kg DM. The concentration of HC on the right side in two samples (0-10 cm) varied from 543.1 to 522.99 g/kg DM. In the soil layers of 10-25 cm, the concentration of HC varied from 516.6 to 43.6 g/kg DM. In soil layer of 15-30 cm, the concentration of HC was about 300.0 g/kg DM. This part of the bog was heavily polluted with the oil (TABLE 3, samples with a letter R). On the left side of the bog in the moss layer of 0-10 cm, the crude oil concentration was 29.0 kg/kg DM in one place and after extraction of this crude oil under room temperature the residual HC concentration became 173.3 g/kg DM. In the other samples, the concentration of HC varied from 508.1 to 567.2 g/kg DM. In the depth of 10-25 cm, the concentration of HC varied from 9.3 to 82.3 g/kg DM. In the soil layer of 25-40cm, the concentration of HC was about 27 g/kg DM. This part of the bog visually seemed a little bit purer than the right one (TABLE 3, samples with letter L).

TABLE 3. CONTENT OF CRUDE OIL AND SATURATED HYDROCARBONS IN MOSS SAMPLES BEFORE AND AFTER AUGMENTATION OF THE BOG WITH THE RHODER

No	Samp-ling depth cm	Before augmentation		After augmentation		Degradation%
		Free crude oil kg/kg	HC, g/kg **	Free crude oil kg/kg	HC, g/kg **	
1_R	(0-10)	15.2	66.9	5.39	105.5	0
	(10-25)	2.94	51.3	2.94	59.8	0
2_R	(0-10)	35.1	73.7	6.37	234.1	0
	(10-25)	*	516.6	*	470.9	8.8
3_R	(0-10)	*	543.1	*	312.4	40.8
	(10-25)	*	84.7	*	45.3	31.2
4_L	(0-10)	*	567.2	*	567.8	0
	(10-25)	*	38.1	*	24.4	35.9
5_L	(0-10)	*	546.7	15.6	230.7	57.8
	(10-25)	*	11.8	*	5.1	56.8
	(25-40)	*	27.1	*	207.6	0
6_L	(0-10)	29.0	173.3	*	47.8	72.4
	(10-25)	*	82.3	*	433.9	0
7_L	(0-10)	*	515.0	*	11.3	97.8
	(10-25)	*	38.8	*	339.7	0
8_R	(0-10)	*	522.9	*	27.6	94.7
	(10-25)	*	43.6	11.4	217.1	0
9_R	(0-10)	25.8	77.8	*	26.5	65.9
	(10-25)	*	76.9	*	260.9	0
10_L	(0-10)	*	508.1	*	8.02	98.4
	(10-25)	*	9.3	7.18	330.2	0
11_R	(0-10)	14.4	280.0	*	190.7	32.1
	(10-25)	*	196.7	4.96	314.1	0
12_R	(0-10)	25.0	187.6	*	123.5	34.2
	(10-25)	*	53.5	8.47	301.5	0
13_R	(0-15)	14.5	290.6	*	318.0	0
	(15-30)	*	308.0	*	384.1	0

Note: R – right side of the bog, **L –** left side of the bog, *** -** free oil is absent; ** - residual saturated HC in the samples after separated the crude oil

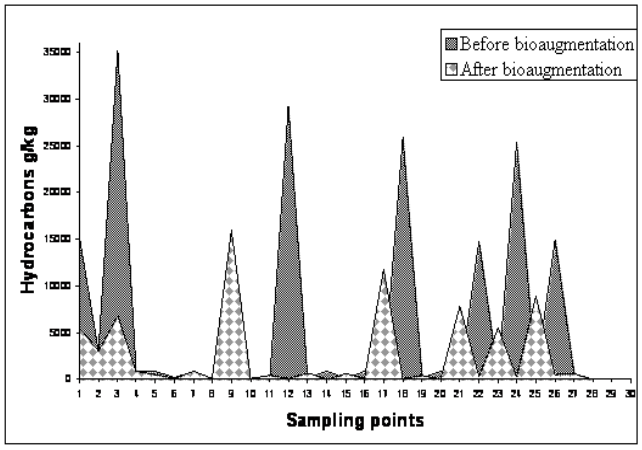

FIGURE 2. BIOREMEDIATION OF THE BOG POLLUTED WITH OIL BY USING THE RHODER

The oil in the samples of the moss which was severely contaminated by the real crude oil (35.1-14.5kg/kg DM), contained the saturated HC of 62.5±1.7%, the aromatic HC of 19.3±1.4%, resins and asphaltenes of 11.8±0.8% and from 5 to 7% of non HC (oxidized substances). Such composition of the oil is a typical for any high quality oil and such oil should be gathered and directed to a refinery plant. Oil contaminating samples of the moss with concentration HC of 850-460 g/kg DM contained the saturated HC of 61.8±1.3%, the aromatic HC of 16.7±0.3%, resins and asphaltenes of 8.7±1.9%. Such contamination also represents the high quality oil and such oil should be gathered as well. The oil in moss samples from the layers of 10-25 cm, 15-30 cm and 25-40 cm contained saturated HC of 49.4±1.12%, aromatic HC of 19.6±2.3%, resins and asphaltenes of 13.4±5.2% and 18% of non HC (oxidized substances). Such HC composition of the pollution indicated that the processes of the oil biodegradation with indigenous anaerobic microorganisms had been inside of these layers. Obtained results showed that the initial huge amount of the crude oil in some places was decreased after bioaugmentation with the Rhoder (TABLE 3 and FIGURE 2), but the oil had appeared in some other places, where previously it was absent. In these places, content of the total saturated HC increased. Probably, such changes in the amount of the crude oil and more impregnation of the top layers (0-10 cm) of the moss with the oil could be due to movement and displacement of the oil because of the small bias to a bulk of the sandy road. Nevertheless,

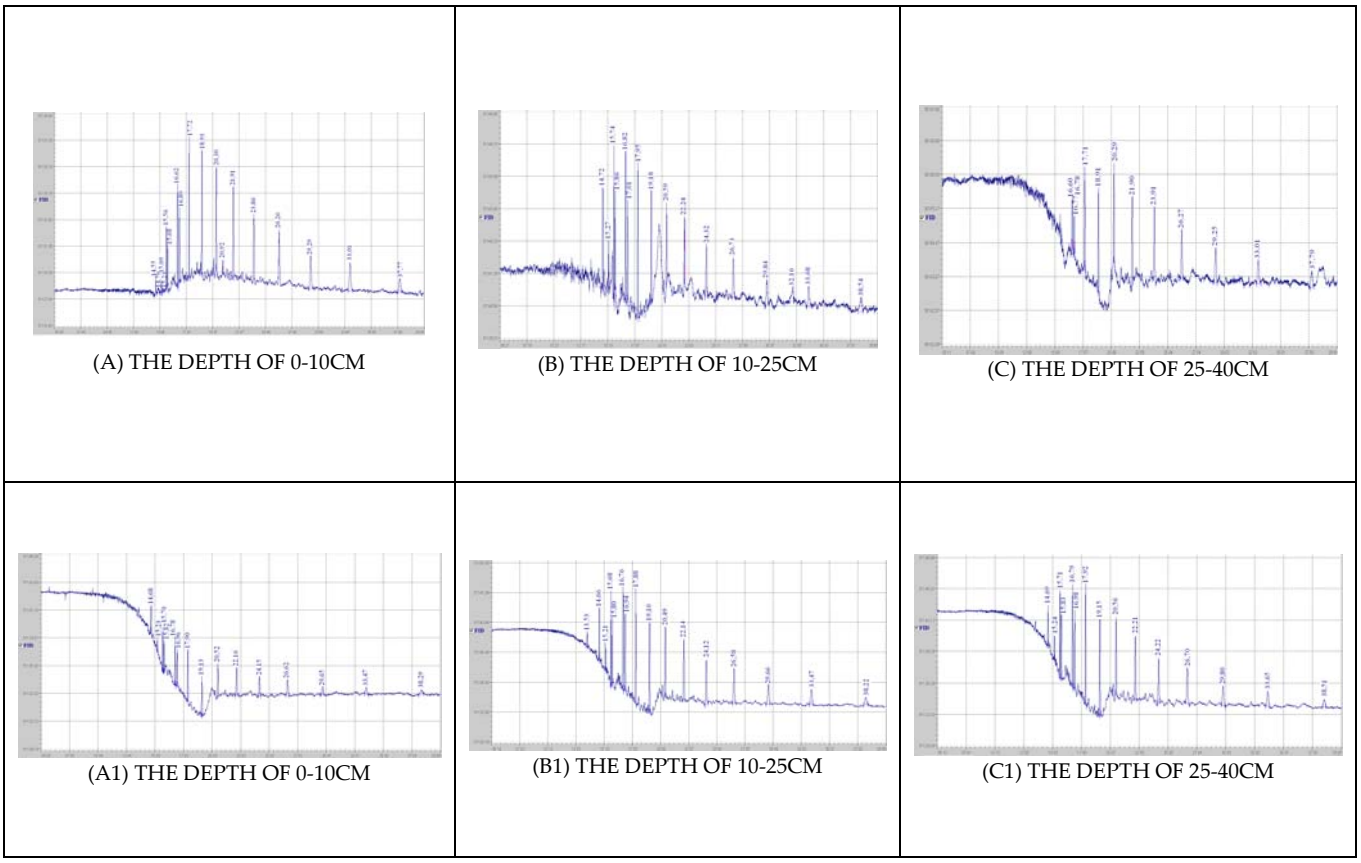

FIG. 3 GC ANALYSIS OF THE MOSS WITH EXTREMELY HIGH OIL POLLUTION, SELECTED FROM THE DIFFERENT DEPTHS BEFORE (TOP) AND AFTER (BOTTOM) AUGMENTATION WITH THE RHODER

positive influence of the Rhoder on the bioremediation of the bog was pronounced. This conclusion was confirmed by results of the HC analysis on GC and HPLC. As for quality oil on a bog surface, of course, it should be collected and transferred to processing, however, it is impossible because of impassable bogs for the equipment of gathering of oil and according to the oil companies, it is economically unprofitable. Chromatograms of HC of the contaminated moss with extremely high and medium levels of the oil pollution before and after bioaugmentation are presented on FIGURE 3 A-C and A1-C1 and confirm results that are described above and below.

After ending of the bioaugmentation with the Rhoder on the right side of the bog there were defined 60.5±0.7% of the saturated HC and 21.5±0.7% of the aromatic HC, and 10.0±0.01% of resins and asphaltenes and about 8% of non HC (oxidized substances) in samples of moss from the depth of 0-10 cm, which initially contained a lot of crude oil. The oil contained saturated HC of 54.0±0.01% and aromatic HC of 19.5±8.5% and resins and asphaltenes of 6.8±0.4% and about 20% of non HC in samples of the soil from layers of 10-25 cm. It is interesting that in the depth of 10-25 cm (anaerobic conditions) degradation process often was more intensive than on the surface of soil. Oil contained 53.5±0.01% of the saturated HC, 23.5±0.01% of the aromatic HC, 11.5±0.01% of resins and asphaltenes and about 13% of oxidized substances in the samples from the moss layer of 25-40 cm. The composition of the oil pollution changed and became the worse if the layer of soil was lower.

Another situation was observed in oil samples from the soil on the left side after ending the bioaugmentation with the Rhoder. The saturated HCs were found of 32.9 ± 5.8% and the aromatic HCs were found of 23.3 ± 1.8% and resins and asphaltenes were found of 29.3 ± 5.9% and oxidized substances were more than 14% in the depth of the moss layers of 0-10 cm, which indicated significant oil oxidizing processes caused by using of the Rhoder.

Oil contained 60.0 ± 1.6% of the saturated HC and 21.0 ± 0.9% of the aromatic HC and 10.8 ± 1.3% of resins and asphaltenes and about 8% of oxygenated compounds in the samples from the moss layers of 10-25 cm (the left side). The quality of oil in 10-25 cm of soil layers was better than that in the upper layers.

Analysis of the residual HC contamination in Hexane fractions by HPLC method in the moss before and

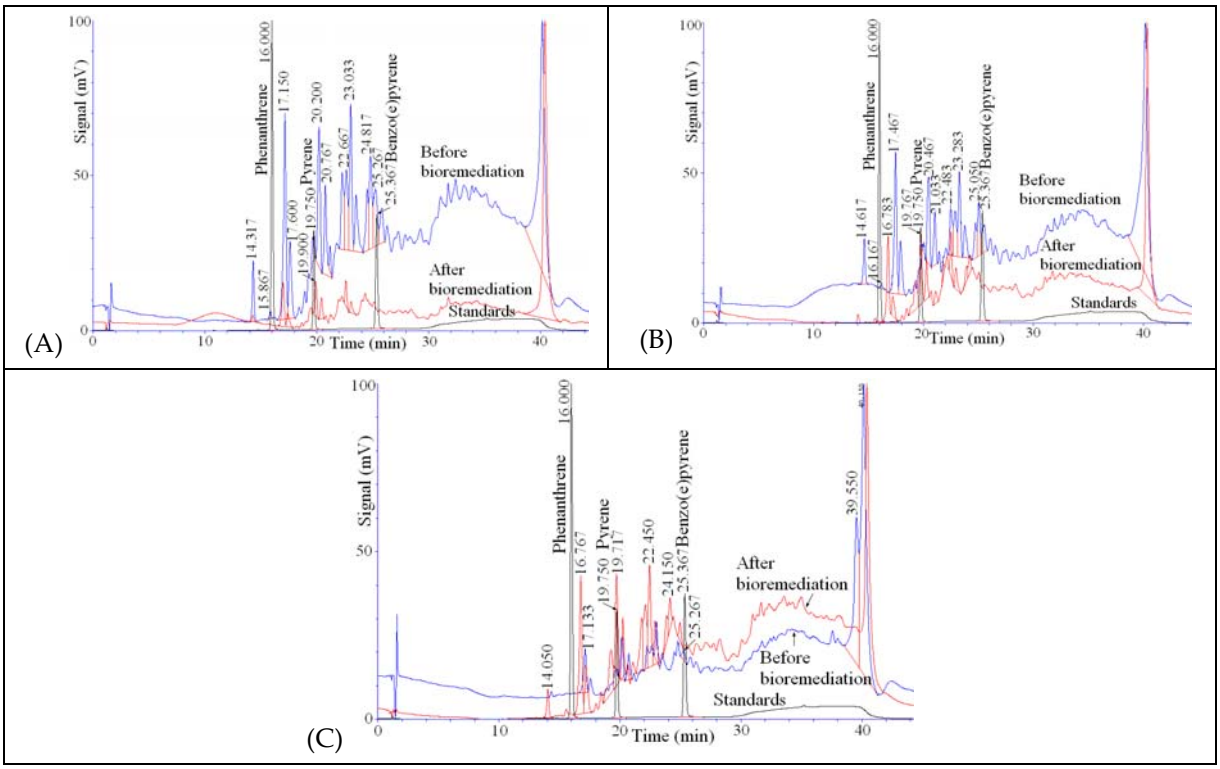

FIG 4 HPLC ANALYSIS OF THE MOSS BEFORE AND AFTER AUGMENTATION WITH THE RHODER (A) FROM THE DEPTH OF 0-10CM; (B) FROM THE DEPTH OF 10-25CM; (C) FROM THE DEPTH OF 10-25CM

after bioaugmentation with the Rhoder showed the oil degradation (FIGURE 4 A-C) in the layers of 0-10 cm and 10-25 cm of the moss (except the layer of 25-40 cm) and confirmed that the degradation of the aromatic HC was observed in these layers of soil. Tentatively, the average efficiency of the Rhoder application can be estimated as 55.2±26.2% for not so favorable weather conditions if an average percentage of oil degradation be calculated (TABLE 3). It is significant that the oil spill on the bog was the fresh (in spring), and the Rhoder was prepared as a liquid concentrate of cells with a high hydrocarbon oxidizing activity ($1.0*10^{11}$ cells per 1 mL of the concentrated product).

Thus, the obtained results have shown, on the one hand, that the Rhoder is able to operate in extreme conditions, such as a super high level of the oil pollution under unfavorable weather conditions without milling of moss that useful for the bioremediation at all. On the other hand, despite of the results described above, there was still a lot of oil on the surface of the bog. Multiple repetition of the bioaugmentation with the Rhoder on the bog heavily polluted with oil will be required for several years to fully restore this bog. The bioaugmentation technology described above cannot be considered as an effective one for the restoration of bogs polluted with oil in severe climatic conditions in the northern part of the Western Siberia. It is necessary to develop a new bioremediation technology with the use of aerobic-anaerobic process of oil biodegradation to avoid the negative influence on the environment that will keep for a long time because of the oil wide spreading contamination far from sites polluted with oil and inpour into the groundwater.

Conclusion

The described bioremediation technology (in-situ) cannot be considered as the effective one for impassable raising bogs polluted with oil behind the Polar Circle in the Western Siberia, because it really requires 3-4 years or more to restore bogs in severe climatic conditions. Nevertheless, the oil-oxidizing preparation Rhoder during in-situ bioremediation is capable to degrade oil (55.2±26.2%) in extreme conditions: a super high level of oil pollution (from 14.4-35.1 kg of oil per kg of dry moss to 516.6 - 43.6g/kg of dry moss) for unfavorable weather conditions without milling which is used for any bioremediation.

Obtained results also showed that the processes of oil biodegradation had been inside of the bottommost layers of the bog due to indigenous anaerobic microorganisms. So it is necessary to develop a new option of bioremediation technology with the

application of aerobic-anaerobic biodegradation of oil for such contaminated bogs which would be more favorable for environment and economically attractive. The Rhoder will be used for project to remedy the upper layer (0-10 cm) of the moss in laboratory conditions on models that simulated a real bog polluted with crude oil. In addition, at the same time, various acceptors of electrons will be forcibly entered into the depth of the models till 40 cm to stimulate the indigenous anaerobic microorganisms capable to degrade oil. Then the best acceptor or acceptors of electrons would been selected and tested at field conditions after several repetitions of such experiments in our laboratory. Devices to perform a new technology in field conditions is planned to be developed.

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